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- (75) Inventors/Applicants (*for US only*): **ROSS, Richard** [GB/GB]; Chemical Sciences, Northern General Hospital, Sheffield S5 7ALL (GB). **ARTYMIUK, Peter** [GB/GB]; University of Sheffield, Dept. of Molecular Biology and Biotechnology, Sheffield S10 2TN (GB). **SAYERS, Jon** [GB/GB]; University of Sheffield, Division of Genomic Medicine Infection & Immunity, Medical School, F Floor, Beech Hill Road, Sheffield S10 2RX (GB).
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- (88) Date of publication of the international search report:
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(54) Title: MULTIMERS OF RECEPTOR-BINDING LIGANDS

(57) Abstract: The invention relates to the provision of oligomeric polypeptides (dimers, trimers, etc) comprising the ligand binding domains of cytokines which are linked via flexible polypeptide linker molecules. The linker molecules optionally comprise protease sensitive sites to modulate the release of biologically active cytokines when administered to a human or animal subject.

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In national Application No

PCT 03/00253

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07K14/52 C12N15/10

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B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, MEDLINE, BIOSIS, EMBASE, CHEM ABS Data, EMBL

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97 23615 A (BUNDESREP DEUTSCHLAND ;BAIER MICHAEL (DE); LANG KURT (DE); METZNER) 3 July 1997 (1997-07-03)	20
Y	the whole document	1,2, 9-19, 21-23, 26-29, 39-42
Y	WEICH N S ET AL: "INTERLEUKIN-3/ERYTHROPOIETIN FUSION PROTEINS: IN VITRO EFFECTS ON HEMATOPOIETIC CELLS" EXPERIMENTAL HEMATOLOGY, NEW YORK, NY, US, vol. 21, no. 5, May 1993 (1993-05), pages 647-655, XP000983864 ISSN: 0301-472X page 650, left-hand column	1,2,5,8, 9,11-15, 18-24, 29,32-42

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☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>LIESCHKE G ET AL: "Bioactive murine and human interleukin-12 fusion proteins which retain antitumor activity in vivo" NATURE BIOTECHNOLOGY, NATURE PUBLISHING, US, vol. 15, no. 1, January 1997 (1997-01), pages 35-40, XP002106574 ISSN: 1087-0156 the whole document</p>	1,2,5,8, 9,11-15, 18-24, 29,32-42
Y	<p>WO 00 37642 A (DAVIS SAMUEL J ;GALE NICHOLAS W (US); STAHL NEIL (US); REGENERON P) 29 June 2000 (2000-06-29)</p> <p>page 26 -page 27; claims</p>	1,2,5,8, 9,11-15, 18-24, 29,32-42
Y	<p>WO 99 52877 A (SMITHKLINE BEECHAM CORP ;ROSENBERG MARTIN (US); WIDDOWSON KATHERIN) 21 October 1999 (1999-10-21) the whole document</p>	16,17, 26-29
Y	<p>US 5 525 491 A (OPPERMANN HERMANN ET AL) 11 June 1996 (1996-06-11)</p> <p>the whole document</p>	1,2,5,8, 9,11-15, 18-24, 29,32-42
Y	<p>WO 92 03569 A (SANGSTAT MEDICAL CORP) 5 March 1992 (1992-03-05)</p> <p>page 4 -page 12; claim 9</p>	1,2,5,8, 9,11-15, 18-24, 29,32-42
Y	<p>WO 01 42298 A (RORNBLUTH RICHARD S.) 14 June 2001 (2001-06-14)</p> <p>the whole document</p>	1,2,5,8, 9,11-15, 18-24, 29,32-42
Y	<p>US 6 037 329 A (CHANDLER LOIS ANN ET AL) 14 March 2000 (2000-03-14)</p> <p>column 32, line 62 -column 33, line 20 column 42, line 54 -column 43, line 44; claim 20</p>	1,2,5, 8-15, 18-24, 29,32-42

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INTERNATIONAL SEARCH REPORT

Ir. International Application No

PCT/83 03/00253

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>GUAN K ET AL: "EUKARYOTIC PROTEINS EXPRESSED IN ESCHERICHIA-COLI AN IMPROVED THROMBIN CLEAVAGE AND PURIFICATION PROCEDURE OF FUSION PROTEINS WITH GLUTATHIONE S-TRANSFERASE" ANALYTICAL BIOCHEMISTRY, vol. 192, no. 2, 1991, pages 262-267, XP009013567 ISSN: 0003-2697</p> <p>-----</p>	

INTERNATIONAL SEARCH REPORT

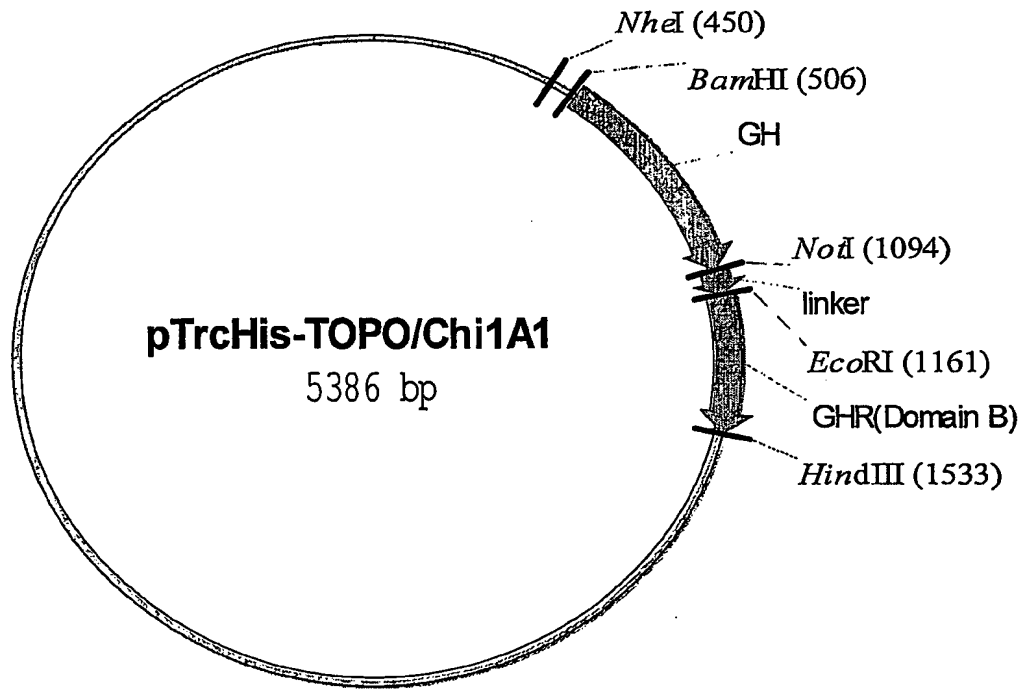
 International Application No
 PCT/JP 03/00253

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9723615	A	03-07-1997	DE 19547933 A1	26-06-1997
			AU 701709 B2	04-02-1999
			AU 1196297 A	17-07-1997
			CA 2240392 A1	03-07-1997
			DE 19681159 D2	01-07-1999
			WO 9723615 A1	03-07-1997
			EP 0870029 A1	14-10-1998
			JP 11503029 T	23-03-1999
			JP 3172968 B2	04-06-2001
			ZA 9610766 A	01-07-1998
WO 0037642	A	29-06-2000	AU 2714700 A	12-07-2000
			CA 2354846 A1	29-06-2000
			EP 1141294 A1	10-10-2001
			JP 2003506008 T	18-02-2003
			PL 349158 A1	01-07-2002
			WO 0037642 A1	29-06-2000
WO 9952877	A	21-10-1999	WO 9952877 A1	21-10-1999
			CA 2328252 A1	21-10-1999
			EP 1071667 A1	31-01-2001
			JP 2002511569 T	16-04-2002
US 5525491	A	11-06-1996	AT 241011 T	15-06-2003
			AU 664030 B2	02-11-1995
			AU 1440392 A	06-10-1992
			CA 2100671 A1	28-08-1992
			DE 69233068 D1	26-06-2003
			EP 0573551 A1	15-12-1993
			JP 6508985 T	13-10-1994
			WO 9215682 A1	17-09-1992
WO 9203569	A	05-03-1992	CA 2090105 A1	01-03-1992
			DE 69132925 D1	21-03-2002
			DE 69132925 T2	10-10-2002
			EP 0547163 A1	23-06-1993
			ES 2171392 T3	16-09-2002
			JP 6502301 T	17-03-1994
			WO 9203569 A1	05-03-1992
			US 5672486 A	30-09-1997
WO 0142298	A	14-06-2001	AU 4016700 A	18-06-2001
			CA 2393659 A1	14-06-2001
			EP 1235853 A1	04-09-2002
			WO 0142298 A1	14-06-2001
US 6037329	A	14-03-2000	US 2002168338 A1	14-11-2002
			US 6503886 B1	07-01-2003
			US 2003040496 A1	27-02-2003
			AU 3374795 A	22-03-1996
			AU 3724495 A	29-03-1996
			AU 710309 B2	16-09-1999
			AU 5862896 A	29-11-1996
			CA 2221269 A1	21-11-1996
			EP 0833665 A1	08-04-1998
			JP 11505805 T	25-05-1999
			WO 9606641 A1	07-03-1996
			WO 9608274 A2	21-03-1996

INTERNATIONAL SEARCH REPORT

International Application No
PCT/B/03/00253

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 6037329	A	WO 9636362 A1	21-11-1996
		AU 702323 B2	18-02-1999
		AU 2272495 A	03-10-1995
		CA 2185671 A1	21-09-1995
		EP 1188448 A2	20-03-2002
		EP 0776218 A2	04-06-1997
		JP 9510352 T	21-10-1997
		WO 9524928 A2	21-09-1995

**FIGURE 1**

Name	5'-Sequence-3'
DiGHEcoF	AGGCGAATTCTTCCCAACCATTCCCTAT (SEQ ID:7)
DiGHNotF	CTTCAAGAGGCGGCGGCCGCTTCCCAACCATTCCCTTAT (SEQ ID:8)
DiGHHinR	TTCCAAGCTTCATCAGAAGCCACAGCTGCCCTCCA (SEQ ID:9)
Lep2TrcFOR	CAAAGCTAGCGGTGGCATGCAAGT (SEQ ID:10)
Lep2TrcREV	AAGCTTGAATTCCTATTACGTCGACTCTAG (SEQ ID:11)
LepLinkFOR	CAGCTGCTGTGGCTTCGGCGGCCGCAGGTGGCGGA (SEQ ID:12)
LepLinkREV	AATGCCTCGAGGAATTCGGAACCTCCG (SEQ ID:13)
Lep2FOR	GGGAAACTCGAGGTGCCCATCCAAAAAGTCCAAGAT (SEQ ID:14)
Lep2REV	GGGAAAGTCGACTCTCTAGAGCACCCAGGGCTGAGGTCC (SEQ ID:15)

FIGURE 2

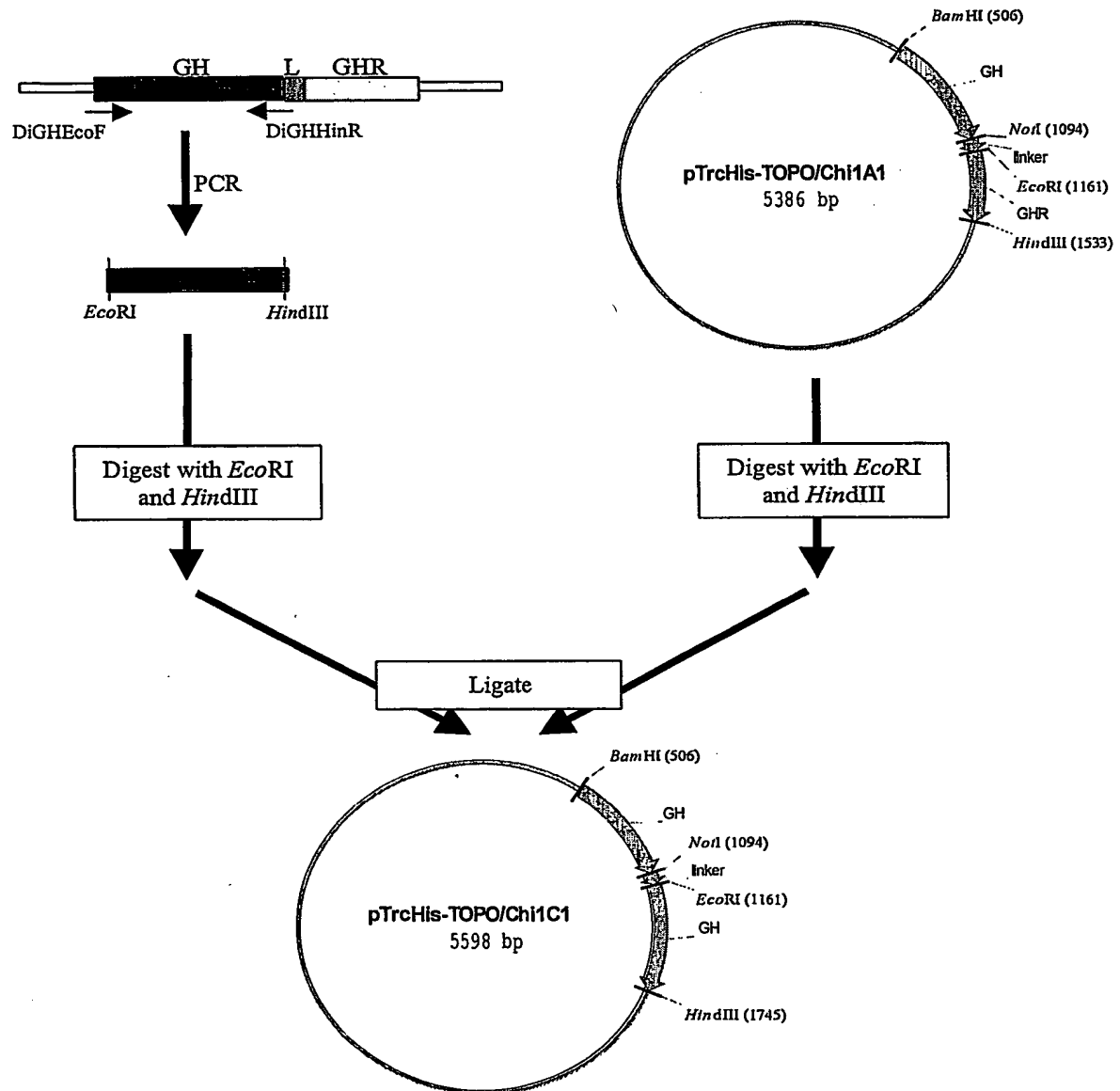


FIGURE 3

Growth Hormone Molecule 1

TTCCCAACCATTCCTTATCCAGGCTTTTTGACAACGCTAGTCTCCGCGC
CCATCGTCTGCACCAGCTGGCCTTTGACACCTACCAGGAGTTTGAAGAAG
CCTATATCCCAAAGGAACAGAAGTATTCATTCTGCAGAACCCCCAGACC
TCCCTCTGTTTCTCAGAGTCTATTCCGACACCCTCCAACAGGGAGGAAAC
ACAACAGAAATCCAACCTAGAGCTGCTCCGCATCTCCCTGCTGCTCATCC
AGTCGTGGCTGGAGCCCGTGACAGTTCCTCAGGAGTGTCTTCGCCAACAGC
CTGGTGTACGGCGCCTCTGACAGCAACGTCTATGACCTCCTAAAGGACCT
AGAGGAAGGCATCCAAACGCTGATGGGGAGGCTGGAAGATGGCAGCCCCC
GGACTGGGCAGATCTTCAAGCAGACCTACAGCAAGTTCGACACAACTCA
CACAACGATGACGCACTACTCAAGAACTACGGGCTGCTCTACTGCTTCAG
GAAGGACATGGACAAGGTCGAGACATTCCTGCGCATCGTGCAGTGCCGCT
CTGTGGAGGGCAGCTGTGGCTTC (SEQ ID:16)

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TTAGTGCCGCGCGGCAGTCCGGGCATTGGCGGCGGTGGCGGC (SEQ ID:17)

Growth Hormone Molecule 1

TTCCCAACCATTCCTTATCCAGGCTTTTTGACAACGCTAGTCTCCGCGC
CCATCGTCTGCACCAGCTGGCCTTTGACACCTACCAGGAGTTTGAAGAAG
CCTATATCCCAAAGGAACAGAAGTATTCATTCTGCAGAACCCCCAGACC
TCCCTCTGTTTCTCAGAGTCTATTCCGACACCCTCCAACAGGGAGGAAAC
ACAACAGAAATCCAACCTAGAGCTGCTCCGCATCTCCCTGCTGCTCATCC
AGTCGTGGCTGGAGCCCGTGACAGTTCCTCAGGAGTGTCTTCGCCAACAGC
CTGGTGTACGGCGCCTCTGACAGCAACGTCTATGACCTCCTAAAGGACCT
AGAGGAAGGCATCCAAACGCTGATGGGGAGGCTGGAAGATGGCAGCCCCC
GGACTGGGCAGATCTTCAAGCAGACCTACAGCAAGTTCGACACAACTCA
CACAACGATGACGCACTACTCAAGAACTACGGGCTGCTCTACTGCTTCAG
GAAGGACATGGACAAGGTCGAGACATTCCTGCGCATCGTGCAGTGCCGCT
CTGTGGAGGGCAGCTGTGGCTTC (SEQ ID:16)

FIGURE 4

Growth hormone molecule 1

FPTIPLSRLFDNASLRAHRLHQLAFDTYQEFEEAYIPKEQKYSFLQNPQT
SLCFSESIPTPSNREETQQKSNLELLRISLLLIQSWLEPVQFLRSVFANS
LVYGASDSNVYDLLKDLEEGIQTLMGRLEDGSPRTGQIFKQTYSKFDTNS
HNDDALLKNYGLLYCFRKDMDKVETFLRIVQCRSVEGSCGF (SEQ ID:18)

Linker region

LVPRGSPGIGGGGG (SEQ ID:19)

Growth hormone molecule 1

FPTIPLSRLFDNASLRAHRLHQLAFDTYQEFEEAYIPKEQKYSFLQNPQT
SLCFSESIPTPSNREETQQKSNLELLRISLLLIQSWLEPVQFLRSVFANS
LVYGASDSNVYDLLKDLEEGIQTLMGRLEDGSPRTGQIFKQTYSKFDTNS
HNDDALLKNYGLLYCFRKDMDKVETFLRIVQCRSVEGSCGF (SEQ ID:18)

FIGURE 5

Leptin molecule 1

GTGCCCATCCAAAAAGTCCAAGATGACACCAAAACCCTCATCAAGACAAT
TGTCACCAGGATCAATGACATTTACACACGCAGTCAGTCTCCTCCAAAC
AGAAAGTCACCGGTTTGGACTTCATTCCTGGGCTCCACCCCATCCTGACC
TTATCCAAGATGGACCAGACACTGGCAGTCTACCAACAGATCCTCACCAG
TATGCCTTCCAGAAACGTGATCCAAATATCCAACGACCTGGAGAACCTCC
GGGATCTTCTTCACGTGCTGGCCTTCTCTAAGAGCTGCCACTTGCCCTGG
GCCAGTGGCCTGGAGACCTTGGACAGCCTGGGGGGTGTCTTGGAAGCTTC
AGGCTACTCCACAGAGGTGGTGGCCCTGAGCAGGCTGCAGGGGTCTCTGC
AGGACATGCTGTGGCAGCTGGACCTCAGCCCTGGGTGC (SEQ ID:20)

Linker region

TTAGTGCCGCGCGGCAGTCCGGGCATTGGCGGCGGTGGCGGC (SEQ ID:17)

Leptin molecule 2

GTGCCCATCCAAAAAGTCCAAGATGACACCAAAACCCTCATCAAGACAAT
TGTCACCAGGATCAATGACATTTACACACGCAGTCAGTCTCCTCCAAAC
AGAAAGTCACCGGTTTGGACTTCATTCCTGGGCTCCACCCCATCCTGACC
TTATCCAAGATGGACCAGACACTGGCAGTCTACCAACAGATCCTCACCAG
TATGCCTTCCAGAAACGTGATCCAAATATCCAACGACCTGGAGAACCTCC
GGGATCTTCTTCACGTGCTGGCCTTCTCTAAGAGCTGCCACTTGCCCTGG
GCCAGTGGCCTGGAGACCTTGGACAGCCTGGGGGGTGTCTTGGAAGCTTC
AGGCTACTCCACAGAGGTGGTGGCCCTGAGCAGGCTGCAGGGGTCTCTGC
AGGACATGCTGTGGCAGCTGGACCTCAGCCCTGGGTGCTTAGTGCCG (SEQ ID:21)

FIGURE 6

Leptin molecule 1

VPIQKVQDDTKTLIKTIVTRINDISHTQSVSSKQKVTGLDFIPGLHPILT
LSKMDQTLAVYQQILTSMPSRNVIQISNDLENLRDLLHVLAFSKSCHL
PWASGLETLDLGGVLEASGYSTEVVALSRLQGSLQDMLWQLDLSPGC (SEQ ID:22)

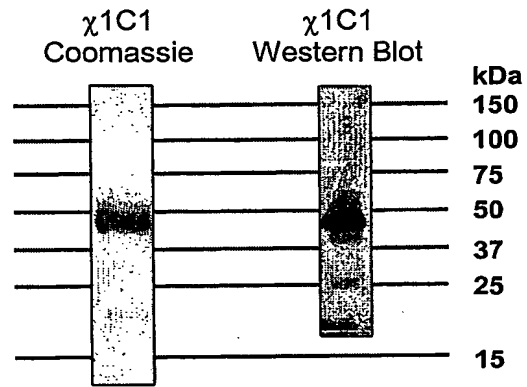
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LVPRGSPGIGGGGG (SEQ ID:19)

Leptin molecule 2

VPIQKVQDDTKTLIKTIVTRINDISHTQSVSSKQKVTGLDFIPGLHPILT
LSKMDQTLAVYQQILTSMPSRNVIQISNDLENLRDLLHVLAFSKSCHL
PWASGLETLDLGGVLEASGYSTEVVALSRLQGSLQDMLWQLDLSPGC (SEQ ID:22)

FIGURE 7

**FIGURE 8**

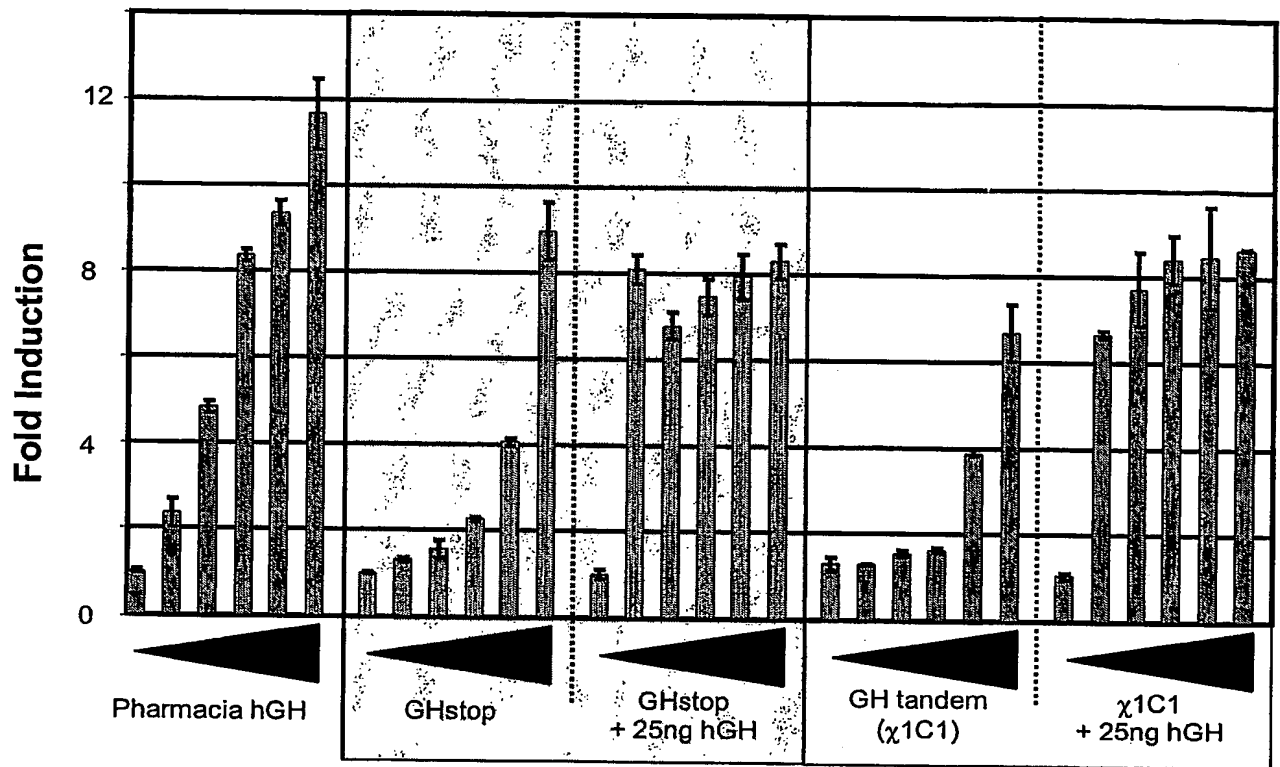


FIGURE 9

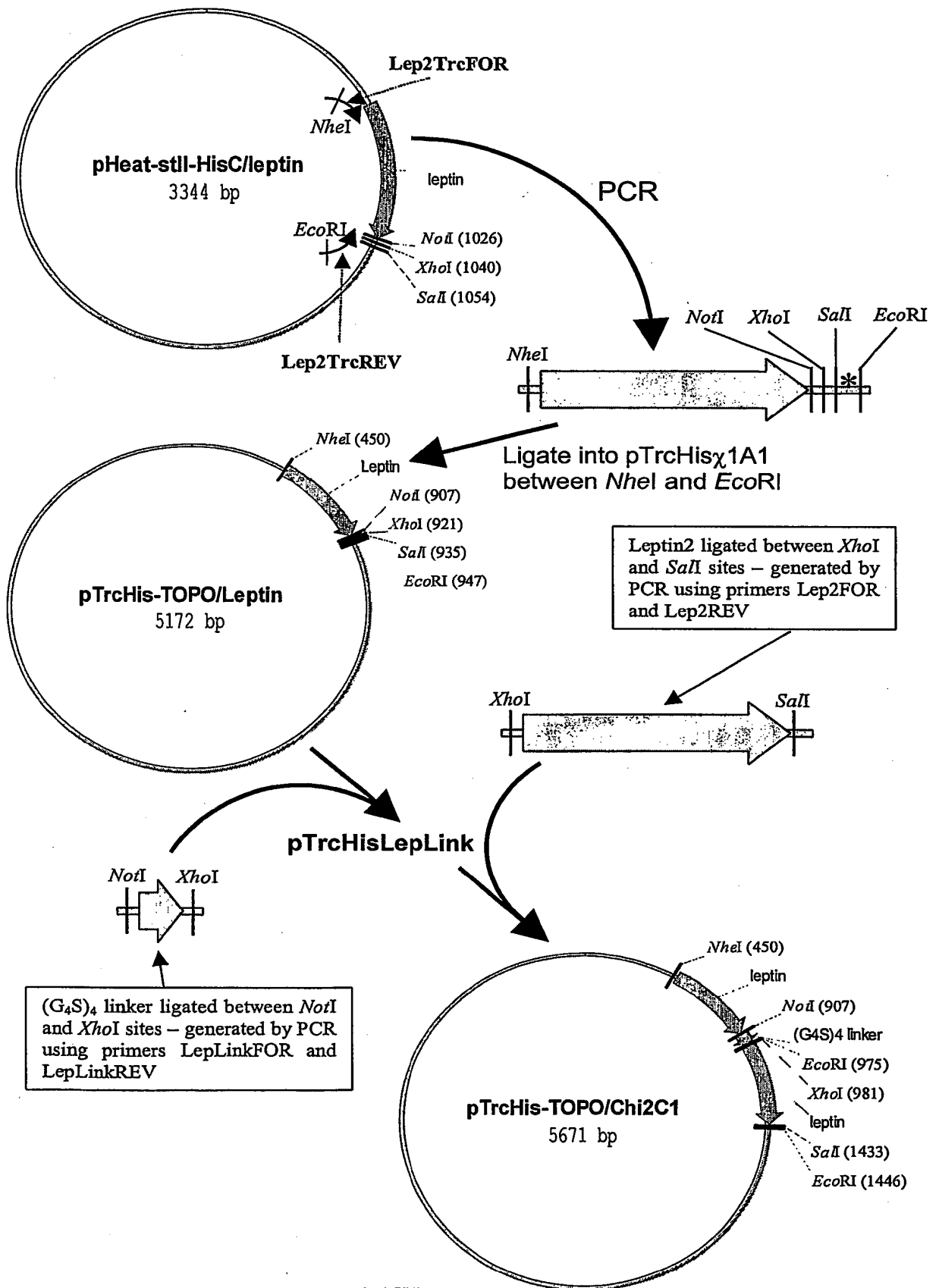
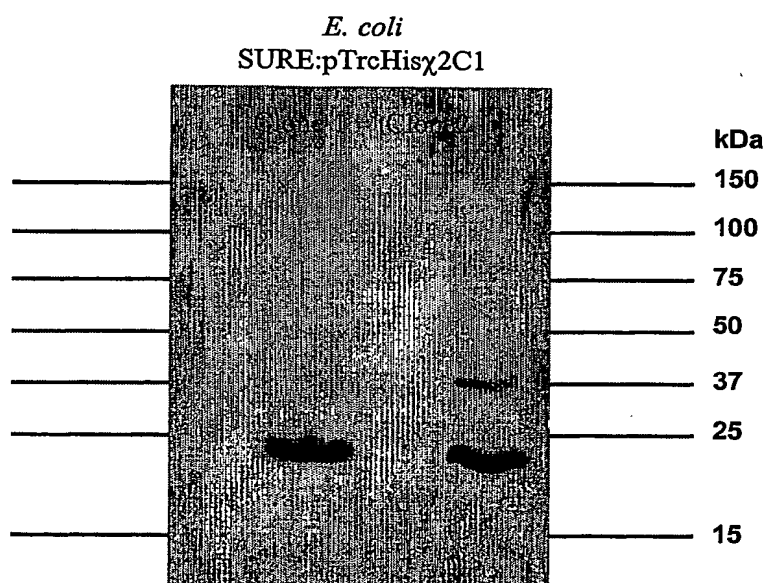


FIGURE 10

**FIGURE 11**

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